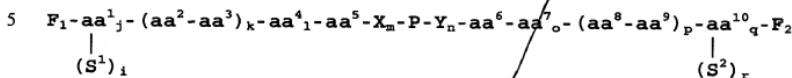


**WHAT IS CLAIMED IS:**

1. A fluorogenic composition for the detection of the activity of a protease, said composition having the formula:



wherein, P is a peptide selected from the group consisting of

10 DEVDGIN (SEQ ID NO:\_\_), (d-O)DEVDGIN (SEQ ID NO:\_\_), DEVDGID (SEQ ID NO:\_\_), LVEIDNG (SEQ ID NO:\_\_), GIETESGV (SEQ ID NO:\_\_), TGRT (SEQ ID NO:\_\_), VMTGRT (SEQ ID NO:\_\_), SEVKLDAEF (SEQ ID NO:\_\_), S(d-E)VK(d-L)DAE(d-F) (SEQ ID NO:\_\_), EDVVCCS (SEQ ID NO:\_\_), EEVEGIN (SEQ ID NO:\_\_), D(d-F)VDGIN, (d-D)EV(d-D)GIN, LVEIENG (SEQ ID NO:\_\_), GIETDSG (SEQ ID NO:\_\_), GIETESG (SEQ ID NO:\_\_), LEHDGIN (SEQ ID NO:\_\_), LETDGIN (SEQ ID NO:\_\_), WEHDGIN (SEQ ID NO:\_\_), YVHDG (SEQ ID NO:\_\_), YVHDGIN (SEQ ID NO:\_\_), YVHDA (SEQ ID NO:\_\_), TGRTG (SEQ ID NO:\_\_), S(d-E)VK(d-L)DAE(d-F) (SEQ ID NO:\_\_), IEPDS (SEQ ID NO:\_\_), PLGIAGI (SEQ ID NO:\_\_), SQNYPIVQ (SEQ ID NO:\_\_);

15

$F^1$  and  $F^2$  are fluorophores and  $F^1$  is attached to the amino terminal amino acid and  $F^2$  is attached to the carboxyl terminal amino acid:

$S^1$  and  $S^2$ , when present, are peptide spacers ranging in length from 1 to about 50 amino acids and  $S^1$ , when present, is attached to the amino terminal amino acid and  $S^2$ , when present, is attached to the carboxyl terminal amino acid:

aa<sup>1</sup> and aa<sup>10</sup> are independently selected from the group consisting of lysine, ornithine and cysteine;

$aa^2$ ,  $aa^3$ ,  $aa^8$ , and  $aa^9$  are independently selected from the group consisting of an amino acid or a dipeptide consisting of Asp, Glu, Lys, Ornithine, Arg

### 30. Citulline, homocitrulline, Ser, homoserine, Thr, and Tyr:

aa<sup>5</sup>, aa<sup>4</sup>, aa<sup>6</sup>, and aa<sup>7</sup> are independently selected from the group consisting of proline, 3,4-dehydroproline, hydroxyproline, alpha aminoisobutyric acid and N-methyl alanine.

X is selected from the group consisting of Gly,  $\beta$ Ala,  $\gamma$ Abu, Gly-Gly.

35 Ahx, C7,  $\beta$ Ala- $\beta$ Gly,  $\beta$ Ala- $\beta$ Ala,  $\gamma$ Abu-Gly,  $\beta$ Ala- $\gamma$ Abu, Gly-Gly-Gly,  $\gamma$ Abu- $\gamma$ Abu, Ahx-

Gly,  $\beta$ Ala-Gly-Gly, Ahx- $\beta$ Ala,  $\beta$ Ala- $\beta$ Ala-Gly, Gly-Gly-Gly-Gly, Ahx- $\gamma$ Abu,  $\beta$ Ala- $\beta$ Ala- $\beta$ Ala,  $\gamma$ Abu- $\beta$ Ala-Gly,  $\gamma$ Abu- $\gamma$ Abu-Gly, Ahx-Ahx,  $\gamma$ Abu- $\gamma$ Abu- $\beta$ Ala, and Ahx-Ahx-Gly;

Y is selected from the group consisting of Gly,  $\beta$ Ala,  $\gamma$ Abu, Gly-Gly, Ahx, C7, Gly- $\beta$ Ala,  $\beta$ Ala- $\beta$ Ala, Gly- $\gamma$ Abu,  $\gamma$ Abu- $\beta$ Ala, Gly-Gly-Gly,  $\gamma$ Abu- $\gamma$ Abu, Gly-Ahx,

5 Gly-Gly- $\beta$ Ala,  $\beta$ Ala-Ahx, Gly- $\beta$ Ala- $\beta$ Ala, Gly-Gly-Gly-Gly,  $\gamma$ Abu-Ahx,  $\beta$ Ala- $\beta$ Ala- $\beta$ Ala, Gly- $\beta$ Ala- $\gamma$ Abu, Gly- $\gamma$ Abu- $\gamma$ Abu, Ahx-Ahx,  $\beta$ Ala- $\gamma$ Abu- $\gamma$ Abu, and Gly-Ahx-Ahx;

when i is 1,  $S^1$  is joined to aa<sup>1</sup> by a peptide bond through a terminal alpha amino group of aa<sup>1</sup>; and when r is 1,  $S^2$  is joined to aa<sup>10</sup> by a peptide bond through a terminal alpha carboxyl group of aa<sup>10</sup>.

10 2. The composition of claim 1, wherein the carboxyl terminal amino acid in which the carboxylic acid group is replaced with an amide.

3. The composition of claim 1, wherein

r is zero; and

aa<sup>10</sup> has a C-terminal amide group or free carboxylic acid group.

15 4. The composition of claim 1, having an amino acid sequence selected from the group consisting of Fa-KDPJGDEVDGINGJPKGY, Fm-KDPJGDEVDGINGJPkamide, Fm-KDPJG(d-O)DEVDGINGJPKGY, Fm-KDPJGDEVDGINGPKG, Fm-KDPGDEVDGINGJPKGY, Fm-KDPJGDEVDGIDGJPkamide, Fm-

KDPJGLVEIDNGPKG, Fm-KDPJGIETESGVGPKG, Fm-KDPJTGRTPKG, Fm-DPTGRTPKG, Fm-KDPVMTGRTPKG, Fm-KDPTGRTPKG, Fm-KDPJGTGRTPKG, Fm-KDPJGTGRTPKG, Fm-KDPGTGRTPKG, Fm-KDPJGSEVKLDAEFGPKG, Fm-KDPJGS(d-F)VK(d-L)DAE(d-F) GC5PKDDY, Fa-KDPJGEDVVCCSGPKG, KDPJGEEVEGINGPKG, KDPJGD(d-F)VDGINGPKG, KDPJG(d-D)EV(d-D)GINGPKG, KDPJGLVEIENGPKG, KDPJGIETDSGPKG, KDPJGIETESGPKG, KDPJGLEHDGINGPKG, KDPJGLETDGINGPKG,

25 KDPJGWEHDGINGPKG, KDPJGYVHDGPKG, KDPJGYVHDGINGPKG, KDPJGYVHDAPKG, KDPJTGRTPKG, KDPCTGRTPKG, KDPCT7GRTPKG, KDPGS(d-E)VK(d-L)DAE(d-F)GPKG, KDPJGIEPDSGPKG, KDPJGPLGAGIGPKG, and KDPJGSQNPYIVQGPKG.

30 5. The composition of claim 1, wherein F<sup>1</sup> and F<sup>2</sup> are the same fluorophore.

6. The composition of claim 5, wherein said F1 and F2 have an excitation wavelength between about 315 nm and about 700 nm.

7. The composition of claim 1, wherein the F<sup>1</sup> molecule is attached through either an  $\alpha$ -amino group of the aa<sup>1</sup> amino acid or through a side chain amino group of the aa<sup>1</sup> amino acid, or through a sulphydryl group of a side chain of the aa<sup>1</sup> amino acid.

5 8. The composition of claim 1, wherein the F<sup>2</sup> molecule is attached either through a side chain amino group of the aa<sup>10</sup> amino acid, through a carboxyl group of the aa<sup>10</sup> amino acid, or through a sulphydryl group of a side chain of the aa<sup>10</sup> amino acid.

9. The composition of claim 1, wherein said fluorophore is selected from the 10 group consisting of rhodamine X, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bis(dimethylamino)xanthylumhalide or other anion (TMR), 9-(2,5 )-dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylum halide or other anion (Rh6G), 9-(2,6 )-dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylum halide or other anion, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bisamino-xanthylum halide or other anion (Rh110), 9-(2,5 15 (or 2,6)-dicarboxyphenyl)-3-amino-6-hydroxy-xanthylum halide or other anion (Blue Rh), carboxytetramethylrhodamine, carboxyrhodamine-X , diethylaminocoumarin, 9-(2,5-dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylum chloride (5-TMR), 9-(2,6-dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylum chloride (6-TMR), 9-(2-carboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylum, 9-(2-carboxyphenyl)-3,6-bis(dimethylamino)xanthylum, and 9-(2-carboxyphenyl)-xanthylum.

20 10. The composition of claim 1, wherein said composition bears a hydrophobic group.

11. The composition of claim 4, wherein said composition bears a hydrophobic group.

25 12. The composition of claim 11, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzylloxycarbonyl, Xanthy (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4= 30 dimethoxybenzhydryl (Mbh), Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzLO), Benzyl

(Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzoyloxycarbonyl (2-Cl-Z), 2-bromobenzoyloxycarbonyl (2-Br-Z), Benzoyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO), t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

- 5 13. The composition of claim 12, wherein said hydrophobic group is Fmoc.
14. The composition of claim 12, wherein said hydrophobic group is Fa.
15. The composition of claim 12, wherein said hydrophobic group is attached to the amino terminus of the molecule.
- 10 16. A method of detecting the activity of a protease, said method comprising contacting said protease with a composition of claim 1.
17. The method of claim 16, wherein said contacting is in a histological section.
18. The method of claim 16, wherein said contacting is in a cell culture.
- 15 19. The method of claim 16, wherein said contacting is contacting a seeded or cultured adherent cell.
- 20 20. The method of claim 16, wherein said contacting is in a cell suspension derived from a biological sample selected from the group consisting of a tissue, blood, urine, saliva, lymph, biopsy.
21. The method of claim 16, wherein said detecting is by a method selected from the group consisting of fluorescence microscopy, fluorescence microplate reader, flow cytometry, fluorometry, absorption spectroscopy.
- 25 22. A method of delivering a molecule into a cell, said method comprising: providing a molecule according to claim 1 attached to a hydrophobic group or to at least one fused ring structure; and contacting said cell with said molecule whereby said molecule enters said cell.

23. The method of claim 22, wherein said hydrophobic group is selected from the group consisting of: selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzoyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4-dimethoxybenzhydryl (Mbh), Tosyl (Tos), 2,2,5,7,8-penta(methyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyl (BzI), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimethyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzoyloxycarbonyl (2-Cl-Z), 2-bromobenzoyloxycarbonyl (2-Br-Z), Benzoyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (CH<sub>2</sub>O), t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

24. The method of claim 22, wherein, said fluorophores are selected from the group consisting of rhodamine X, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bis(dimethylamino)xanthylumhalide or other anion (TMR), 9-(2,5 )-dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylum halide or other anion (Rh6G), 9-(2,6 )-dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylum halide or other anion, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bisamino-xanthylum halide or other anion (Rh110), 9-(2,5 (or 2,6)-dicarboxyphenyl)-3-amino-6-hydroxy-xanthylum halide or other anion (Blue Rh), 20 carboxytetramethylrhodamine, carboxyrhodamine-X , diethylaminocoumarin, 9-(2,5-dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylum chloride (5-TMR), 9-(2,6-dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylum chloride (6-TMR), 9-(2-carboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylum, 9-(2-carboxyphenyl)-3,6-bis(dimethylamino)xanthylum, and 9-(2-carboxyphenyl)-xanthylum..

25. The method of claim 22, wherein, said fluorophores are selected from the group consisting of: of carboxytetramethylrhodamine, carboxyrhodamine-X and diethylaminocoumarin.

26. The method of claim 22, wherein, said cell is a mammalian cell.